ISOLATION OF BIOMOLECULES

The present invention relates to a purification method and apparatus for use in obtaining samples of biomolecules. The invention relates more particularly, but not exclusively, to such method and apparatus which may be used for obtaining samples of nucleic acids or proteins.

Numerous methods are known for obtaining biomolecules, for example nucleic acids and proteins, from biological material such as viruses, bacterial and eukaryotic cells, cell aggregates and tissue or body fluids. Typically the biomolecule to be obtained is a soluble molecule and is "released" from the biological material by a lysis procedure (e.g. alkaline lysis) resulting in a suspension comprised of a solution of the target biomolecule also containing soluble proteins, carbohydrates, fats, amino acids and other metabolites from the disrupted cells.

In many methods known in the art for effecting the purification of a desired biomolecule, those molecules which would otherwise contaminate the desired product are insoluble, or are rendered so by a chemical process. The insoluble material is then removed by methods known in the art (e.g. centrifugation and aspiration of the supernatant) to achieve a degree of purification of the soluble material. These insoluble materials may include, for example, whole cells, or fragments thereof, flocculated proteins and unwanted nucleic acid material (e.g. chromosomal DNA contamination of a plasmid DNA preparation).

After separation of the insoluble material, from the solution, the latter is applied to a solid phase binding matrix under conditions (e.g. in the presence of a chaotropic salt) such that the matrix binds the biomolecule of interest. Subsequently the solution is removed from the matrix (leaving the biomolecule bound thereto), the matrix washed to remove non-bound material, and the biomolecule eluted.

The separation of the insoluble material from the solution is generally effected by centrifugation of the suspension followed by careful pipetting. However the use of centrifugation is a disadvantage in that it does not easily allow the procedure for obtaining the biomolecule to be fully automated. Furthermore, the separation of soluble from insoluble material by pipette is awkward to perform and must be carried out accurately to prevent unwanted insoluble material from being added to the solid phase matrix and causing contamination.

One purification method which seeks to overcome some of the problems associated with conventional techniques is disclosed in WO-A-95/02049. The apparatus disclosed involves a pneumatic delivery system which is used to add, mix and remove reagents from a flow-through vessel in order to separate a target biomolecule from cells. The vessel has two chambers between which is a porous membrane or filter. The membrane functions to retain cells and cellular debris and insoluble material in the upper chamber whilst soluble material is filtered through and further purified by binding to a solid phase matrix present in the lower chamber.

There are however disadvantages associated with the technique disclosed in WO-A-95/02049. In particular, if it is desired to treat the solid phase matrix (with biomolecule bound thereto) with additional solutions (e.g. wash or elution solutions) it is necessary to pass those solutions into the lower chamber either via the upper chamber and filter (which retains insoluble material) or provide additional inlets in the lower chamber. In the former case, there is the risk of contamination. In the latter case, the provision of additional ports in the lower chambers makes the apparatus difficult to manufacture.

It is an object of the present invention to obviate or mitigate the aforementioned disadvantages.

According to a first aspect of the present invention there is provided a method of obtaining a sample containing the biomolecule from a suspension comprising a solution of the biomolecule and insoluble material, the method comprising the steps of:

(a) providing a biomolecule purification assembly comprised of a vessel having a liquid inlet and of a filter unit removably located on the liquid inlet;

(b) effecting a filtration of the suspension through the filter unit so as to cause the solution to enter the vessel through the liquid inlet;

(c) removing the filter unit from the liquid inlet;

(d) immobilising the biomolecule on a solid phase support; and

(e) subjecting the molecule to at least one of the steps of washing on the support and elution from the support to obtain a purified sample of the biomolecule.

According to a second aspect of the present invention there is provided apparatus for obtaining a sample of a biomolecule from a suspension comprising a solution of the biomolecule and insoluble material, the apparatus comprising

(i) a biomolecule purification assembly comprised of a vessel having a liquid inlet and of a filter unit removably located on the liquid inlet of the vessel;

(ii) means for causing the solution present in the suspension to pass through the filter unit and the liquid inlet into the vessel; and

(iii) means for removing the filter unit from the vessel.

According to a third aspect of the present invention there is provided a biomolecule purification assembly comprising a vessel having a liquid inlet and a filter unit removably located on the liquid inlet wherein the vessel is in the form of a column having a first bore section and a second bore section of reduced diameter as compared to the first section, and the filter unit is comprised of a sleeve which houses the filter and which is removably located on the end of the vessel remote from the first bore section.

In accordance with the invention therefore separation of insoluble material from the suspension is effected through a filter unit which is removably located in the liquid inlet of a vessel and a filtration is effected causing the solution to enter the vessel. Any insoluble impurities in the suspension are retained on the filter unit. Subsequently, the filter is removed from the inlet and is preferably discarded rather than being reused. Thus each filter need only be used once avoiding problems of contamination.

The method of the invention negates the requirement for the suspension to be centrifuged to separate soluble and insoluble materials in the suspension. The method further negates the requirement for accurate separation of the soluble and insoluble phases by use of a pipette. Furthermore, the method of the invention is relatively simple to perform and is eminently suited to automation as will be appreciated from the description given below.

It is preferred that, in step (e) of the method, the step of washing or elution is effected on solid phase support contained within the vessel. Preferably both of the steps of washing and elution are effected, and the biomolecule of interest is eluted through said inlet of the vessel.

It is particularly preferred that the solid phase support material is a particulate or bead-like material which is introduced into the vessel (preferably through said inlet) after the step of removing the filter unit therefrom.

The vessel of the biomolecule purification assembly is preferably a flow through vessel, most preferably an open-ended column, e.g. having a volume sufficient to hold 0.25 to 1.5ml of sample, disposed vertically so that its lower end provides the aforementioned liquid inlet and the upper end may be used for the introduction of additional reagents into the column as required.

The vessel may comprise upper and lower bore sections whereof the diameter of the upper section is greater than that of the lower section, the two sections being connected by a intermediate, tapering bore section, the purpose of the reduced section lower bore will be described below.

The filter unit may be of any material capable of tolerating the reagents used and will comprise a filter element having a pore size which is capable of preventing passage of the insoluble material of the composition therethrough but which is not so small that the flow rate through the filter becomes unacceptably low. Typically the pore size of the element will be in the range 0.2 to 50 microns.

The filter unit may, for example, comprise a sleeve or the like for location over the liquid inlet of the vessel and a filter located in the sleeve. The filter unit may for example be a push fit or a loose snap fit over the liquid inlet.

Preferably the filter incorporates a depression locating in close proximity to the liquid inlet of the vessel. This allows filtrate to come into close proximity with the inlet and enhance the rate of filtrate uptake into the vessel.

A preferred embodiment of filter unit (which also provides, in its own right, a fourth aspect of the invention) comprises

(a) an elongate sleeve having one end (the "suspension inlet end") through which a suspension to be filtered enters the sleeve and an opposite end for location over a column of a biomolecule purification assembly, said sleeve having a first body portion and a second body portion which internally tapers from the first body portion to said suspension inlet end, and

(b) a filter having a head portion locating in, and occupying the cross-section of, the first body portion, and a frustoconical or conical body portion extending into said second body portion of the sleeve and tapering therein at a larger angle than the internal taper of the sleeve.

The construction of filter unit as defined in the previous paragraph has a number of advantages as detailed below.

Firstly, there is clearance between the outer surface of the conical or frustoconical portion of the filter and the inner wall of the tapering portion of the sleeve. This ensures that there is a relatively large area of the filter exposed to the suspension to be filtered so that the filter unit has as high a capacity for filtration as possible given the overall dimensions of the filtration unit.

Secondly, the "dead space" within the filter unit (i.e. the space between the outer surface of the conical or frustoconical portion of the filter and the tapering section of the sleeve) may be relatively low whilst still allowing the advantage of the previous paragraph. The minimum dead space ensures that there is a minimum liquid remaining (in the dead space) when all of the suspension to be filtered has been drawn up into the filter unit and air is about to enter the column.

Thirdly, the elongate nature of the filter unit ensures that it's lowermost, suspension inlet end may be juxtaposed to the base of a microtitre well or the like.

Overall, therefore, the filter unit allows maximum amount of suspension to be filtered, with high filtration efficiency, before air enters the unit.

Preferably the first body portion of the sleeve is of circular internal cross-section and the head of the filter is also cylindrical so as to be a close fit therein.

The filtration step of the method of the invention may be effected in a number of ways. Thus for example, a reduced pressure may be applied to the interior of the vessel causing solution from the suspension to be drawn through the filter unit into the vessel.

As an alternative to the use of reduced pressure, the suspension may be provided in an open-topped container within which the filter unit is a close sliding fit such that by moving the filter unit within the container towards the base thereof solution is caused to be forced through the filter unit into the vessel for binding of the biomolecule as described above.

Subsequent to the filtration operation, the filter unit is removed from the vessel.

It is particularly preferred in accordance with the invention that the suspension to be filtered is contained in a well and that once the filtration operation is complete the filter unit is automatically discarded into that well. This may be achieved in a number of ways. Thus, for example, the biomolecule purification assembly may be lowered towards the well to effect filtration and move upwardly after filtration is completed and the apparatus may incorporate a stripping arrangement which acts on the filter unit as the biomolecule purification assembly is moved upwardly causing the filter unit to be discharged into the well. Alternatively, the filter unit and microtitre

well have inter-engagable formations whereby as the biomolecule purification assembly is lowered towards said well the formations come into an engaging relationship requiring a greater force to release the engagement than is required for removing the filter unit from the vessel. Thus, as the vessel is moved upwardly away from the well, the engagement is maintained and the filter unit is removed from the vessel and retained in the well.

Subsequent to the removal of the filter unit, the biomolecule is immobilised in a solid phase support (i.e. step (d) of the method), preferably in the form of particles or beads having a cross-sectional size (e.g. diameter) of 0.05 to 250 microns (e.g. 0.1 to 250 microns). Preferably the particles do not fill the entire space between the retaining means so that the particles may be "fluidised" within the vessel.

The immobilisation of the biomolecule onto the solid phase support is preferably effected in the presence of a binding agent composition. In the case that the biomolecule to be immobilised is a nucleic acid, the binding agent composition may for example comprise polyethyleneglycol together with sodium and potassium cations and chloride and acetate anions. Alternatively the binding agent formulation may comprise a chaotropic salt or other agents capable of effecting absorption of the biomolecule onto the support. By chaotropic salt it is meant any substance capable of altering the secondary, tertiary and/or quaternary structure of a protein or nucleic acid molecule, but leaving at least the primary structure intact. Examples of chaotropic salts which may be utilised to allow binding of nucleic acid or proteins to the solid phase binding matrix are guanadinium salt, sodium iodide, potassium iodide, sodium (iso)thiocyanate, urea or combinations thereof. Preferred chaotropic salts for use in the present invention include guanidinium hydrochloride and guanidinium (iso)thiocyanate. For the purposes of effecting step (d) of the method (i.e. immobilising the biomolecule on the solid phase support), the filtrate in the vessel may contain the chaotropic salt or other immobilising agent(s) and the filtrate is discharged onto the support material prior to the resultant mixture being taken back into the

vessel. Alternatively, the filtrate may be discharged into a mixture of the support and the binding agent composition and the resultant mixture is then taken back into the vessel.

Subsequently, the mixture of the filtrate, binding agent composition and the beads may be drawn back up into the vessel and, if desired, may be subjected to at least one cycle of discharge from, and uptake back into, the vessel to improve mixing.

It is preferred that the support comprises magnetic beads.

The magnetic beads within the vessel may be manipulated by a magnet positioned externally of the vessel.

Step (e) of the method may be effected in a number of ways.

Thus, for example, a magnet may be used to "hold" the magnetic beads (with immobilised biomolecule) at the side of the vessel. In the case of the preferred biomolecule purification assembly in which the vessel has a reduced diameter bore section, it is preferred that the beads are "held" at this position in the vessel.

Subsequently, the solution may be discharged from the vessel, wash buffer introduced into the vessel, and the magnet manipulated to aid re-suspension of the beads in the buffer. The magnet may once again be used to hold the beads in place and the wash buffer discharged from the vessel.

The washing operation may be repeated at least once. For preference the final wash step employs an aqueous solution of ethanol (preferably containing more than 60%v/v ethanol).

After removal of the final wash solution from the vessel, (and with the beads being held therein by means of the magnet), air may be passed over the beads to effect drying. Alternatively or additionally heat may be applied to the vessel, e.g. by locating the latter in a heating block.

Subsequently, the biomolecule may be eluted from the vessel. This may be achieved by introducing an elution buffer into the vessel, admixing the particles with the buffer, heating the admixture, immobilising the particles by means of the magnet, and discharging the solution (containing dissolved biomolecule) from the vessel for collection and subsequent processing

A particularly preferred embodiment of apparatus in accordance with the invention is capable of handling an array of biomolecule purification assemblies and therefore each of the individual steps (described above) of filtration, removal of the filter unit, "pick up" of magnetic particles, washing and elution is effected simultaneously on all members of the array.

In such an apparatus, it is preferred that the vessels (of the biomolecule purification assemblies) are vertically disposed, open-ended columns. The upper ends of such columns may be associated with pumps for applying reduced pressure to the interior of the column for drawing liquid into the columns (e.g. for the purposes of the filtration and washing and elution operations) and for blowing or drawing drying air through the columns. Furthermore, the upper ends of the vessels may be associated, via appropriate valving arrangements, with appropriate reagent reservoirs permitting reagents to be passed downwardly into the columns if required.

A particularly preferred embodiment of apparatus in accordance with the invention for use in conjunction biomolecule purification assemblies as defined in the previous paragraph comprises separate filtration, bead "pick up", washing and elution stations Thus, at the filtration station, there may be a first set of wells (e.g. a

BioBlock) each containing an aliquot of the suspension and each member of the array effects filtration of an aliquot from the corresponding well at the station. The apparatus may be such that the filter unit of the biomolecule purification assembly is discharged into the respective well at the filtration station. At the bead "pick up" station, there may be a second set of microtitre wells each containing magnetic beads in a solution of a binding agent. At the wash station, wash solution may be passed to the vessel (e.g. by pumping) and discharged through the liquid inlet into a waste receptacle which may be connected to a drain. Furthermore, the elution station may have a second set of microtitre wells containing elution buffer which is taken into the column and mixed with the beads. The final step of elution is then effected by holding the magnetic beads in position and discharging the liquid containing the desorbed biomolecule from the vessel. Obviously, the apparatus will include a magnet or magnets as necessary for manipulating the magnetic beads.

In a particularly preferred embodiment of the apparatus as described in the previous paragraph, the apparatus incorporates a head arrangement capable of selectively "picking-up" and releasing 12 of the biomolecule purification assemblies. Furthermore, the wells at the various stations may each be provided by a 12 by 8 array of microtitre wells so that in any one cycle of the apparatus a total of 12 samples may be processed. By operating the apparatus through 8 cycles then a total of 96 samples may be processed before the microtitre wells need to be replaced.

It is also preferred that the apparatus incorporates an upstream station at which the head arrangement is capable of "picking-up" the biomolecule purification assemblies to be used in any one cycle of the apparatus and a final discharge station at which the vessels of the assemblies are discarded. Thus, if the head arrangement is capable of holding 12 biomolecule purification assemblies then 12 such assemblies are "picked-up" at the beginning of each cycle of the machine.

Such an apparatus will further comprise mechanisms for moving those parts of the apparatus as are required to complete the method of the invention in the directions (X,Y and/or Z) so to do. The apparatus as described above may readily be automated and is capable of operating under the control of a programmed microprocessor.

It will thus be appreciated that a preferred embodiment of apparatus will comprise at least the features of

(a) a head arrangement having a plurality of individual column supporting heads on which the upper ends of disposable columns may be removably mounted and which have fluid flow passageways for transfer of fluids into and out of the upper ends of the columns;

(b) means for moving a supply of disposable columns so that upper ends of columns to be mounted on the supporting heads are presented below said heads;

(c) means for moving the head arrangement relatively downwardly towards the tops of the columns whereby the upper ends thereof become removably mounted on said head, and for moving the head arrangement relatively upwardly with columns mounted thereon in readiness for subsequent stages of the process.

(d) reservoirs for reagents/wash solutions as appropriate;

(e) means (e.g. for providing pressure variation in the columns) for causing liquid samples, liquid extracts or mixtures of such liquids with reagents thereof, provided at the lower tips of the columns (e.g. in wells of a microlite plate, BioBlock or similar) to be drawn upwardly into the columns and discharged from the lower tips thereof for processing steps as required to obtain the desired biomolecule; and

(f) means for removing the columns from the heads for disposal.

Such an apparatus is referred to herein as an "apparatus of the kind defined".

The apparatus of the kind defined may be operated in accordance with the sequence of processing steps as described more fully above. As a variation of that sequence (in which the disposable column is mounted on the column supporting head with removable filter attached to the column) the disposable column may initially be mounted on the supporting head and the filter subsequently mounted in the lower end of the column using the means (b) and (c) operated as appropriate.

The method and apparatus of the invention are particularly suitable for obtaining a sample of a biomolecule from a suspension obtained by a lysis procedure (e.g. a standard alkaline lysis procedure as well known to those skilled in the art) effected on a biological material.

The target biomolecule may, for example, be a nucleic acid (DNA or RNA) and may for example be a semi-purified or non purified, native or synthesised nucleic acid. The target soluble biomolecule may be any DNA or RNA sequence from a viral, bacterial, animal or plant source. Apart from the utility in purifying DNA and RNA samples and especially for purifying plasmid DNA and other recombinant DNA constructs, such as phagemids, free from chromosomal DNA, the method and apparatus according to the present invention are also suitable for isolating recombinant proteins and antibodies, especially from cellular samples

The biological material on which the lysis is effected may for example comprise cells. For the purposes of the present specification, the term "cell" is intended to encompass bacterial cells, cells (e.g. blood cells) from higher organisms, virus particles and other cell types or organelles which contain the target biomolecule and which may be released in a soluble form by a lysis procedure.

In the case of bacteria, the nucleic acid to be isolated may be from the bacterial genome or from an extragenomic element such as a plasmid. Phage infected bacteria may also be used for the preparation of phage DNA, such as M13 DNA.

According to a preferred embodiment of the present invention the composition from which the target biomolecule is to be isolated is a lysed and neutralised bacterial cell composition containing soluble plasmid DNA and insoluble precipitated genomic DNA, flocculated protein and other cellular debris.

In order to isolate plasmid DNA that has been propagated within bacterial hosts, the following steps may be followed:

i) growth of bacterial host in an enriched medium;

ii) centrifugation of bacteria to form a pellet after which the supernatant is discarded;

iii) resuspension of the bacterial pellet in a buffered solution;

iv) addition of lysis reagent which releases the cellular contents; and

v) addition of neutralisation solution which causes the formation of suspension comprising a solution containing dissolved plasmid.

In a particularly advantageous implementation of the invention, the resuspension obtained from step (iii) may be provided, e.g. in a microtitre well, to the apparatus which is adapted to be such as to add lysis solution to the resuspension. In the preferred embodiment of the invention, in which the vessels (of the biomolecule purification assemblies) are vertically disposed, open-ended columns, the apparatus comprises a 'column-head' on which the biomolecule purification assemblies are to be mounted. However, prior to mounting of the assemblies on their respective heads, lysis solution may be injected ("fired") from the head into the resuspension in the micro-titre well. The neutralising solution may be added in the same way. This will also provide for good mixing of the lysis solution and neutralising solution with the

resuspension. Subsequently, the biomolecule purification assemblies are mounted on their respective heads for effecting separation, washing and elution procedures as described.

In an alternative implementation, the lysis solution (drawn from a reservoir thereof) may be ejected downwardly through the column into the resuspension prior to mounting of the filter unit on the column.

In both cases the inventors have found that introduction of the lysis solution and neutralising solution in this way into the resuspension can avoid the need for any further mixing provided that sufficient time is allowed for lysis and neutralisation to be effected. Alternatively agitation may be employed (e.g. a shaking platform) to speed lysis/neutralisation. Furthermore, the introduction of the lysis and neutralising solution is effected without the dispensing apparatus coming into contact with the sample or its containing vessel.

Either single or multiple aliquots of lysis solution and neutralising solution may be discharged into each well. In a particularly convenient form of the apparatus, there is a single row of n (e.g. 12) column supporting heads and the cell samples to be processed are held in wells of a microtitre plate or similar in which the wells are arranged as m rows each of n wells (i.e. an m x n array, e.g. 8 x 12). The use of multiple aliquots of lysis solution or neutralising solution will provide for better mixing (or at least more efficient extraction of products) than a single addition of reagent. In such an apparatus, the samples in the n wells of any one of the m rows are simultaneously treated with lysis reagents (by downward ejection from the n supporting heads). It will generally be preferred that the lysis reagents are discharged into all wells of the (m x n) array prior to the disposable columns being mounted on the heads for subsequent steps of the extraction procedure. Thus, lysis agents may initially be added to the n wells of the first of the m rows of the microtitre plate or similar, subsequently the plate is moved relative to the heads so that the second of the

m rows is below the heads for introduction of lysis reagents into these wells and so on until the m rows of wells have been treated with the lysis solution. If desired, the above procedure may then be repeated for the introduction of neutralising solution (via the heads) into the wells. The disposable columns may then be mounted on the supporting heads and all steps of the extraction procedure effected for the samples in the first row of wells prior to replacement of the columns, processing of the second row and so on until all wells of the (m x n) array have been processed.

In a modification of the procedure, columns may be mounted on the heads prior to dispense of the lysis and/or neutralisation reagents.

The time for which the lysis reagents are dispensed into each of the wells may for example be 0.1 to 1 seconds although this will depend in volume to be dispensed (typically 150µl of lysis solution and 150µl neutralisation solution) and pump speed. The discharge outlet of the column supporting head may be about 40mm to 100mm (preferably about 70mm) above the surface of the sample into which the lysis reagents are to be discharged. Typically also the discharge outlet of each supporting head will have a diameter of 0.1 to 1 mm (preferably about 0.75mm).

It should however be noted that all of the values set out in the previous paragraph are exemplary and other values, which may readily be evaluated, may be required depending on the actual construction of the apparatus.

The invention will be further described, by way of example only, with reference to the accompanying drawings, in which:

Fig. 1 schematically illustrates a first embodiment of the invention;

Fig 2 schematically illustrates the column of the assembly shown in Fig 1;

Fig 3 illustrates the sleeve of the filter unit in Fig 1;

Fig. 4 schematically illustrates to an enlarged scale the filter of the assembly shown in Fig 1;

Fig 5 is an exploded view, to an enlarged scale, of a biomolecule purification assembly incorporating a filter unit in accordance with a further embodiment of the invention;

Fig 6 illustrates to a still further enlarged scale, the filter incorporated in the filter unit illustrated in Fig 5;

Fig 7 illustrates a modification of the filter unit shown in Fig 6; and

Figs 8 and 9 schematically illustrate one embodiment of process in accordance with the invention.

Referring firstly to Fig. 1, there is illustrated a biomolecule purification assembly 1 for use in obtaining a purified sample of a biomolecule of interest from a suspension (e.g. as obtained by an alkaline lysis procedure) comprising a solution of the biomolecule containing insoluble biological debris. The illustrated assembly 1 comprises a vertically disposed, open-ended column 2 and a filter unit 3.

Referring to Fig 2, column 2 is referenced for convenience as being comprised of body sections 4, 5, 6 and 7. Body section 4 defines an upper cylindrical bore 4a which at its lower end is connected to a downwardly tapering section 5a leading into a lower bore 6a which is of reduced diameter as compared to bore 4a. At its lower end bore 6a leads into a tapering section 7a defined within the lower section 7 of the column 2.

The lower end of tapering section 7a defines a liquid inlet 8 for the column.

At the upper end of column 2 there is provided a formation 9 by means of which the column may be mounted on the head of a sample processing apparatus of the type described more fully above.

Referring back to Fig 1, the filter unit 3, is a two component part and comprises a sleeve 10 (see Fig 3) having an internal bore 11 within which is housed a filter 12(see Fig 4) having depression 13 in its upper surface. Filter 11 is such that it is permeable to liquids but is capable of filtering the insoluble debris in the suspension from which the biomolecule is to be obtained.

Sleeve 10 is removably mounted on column 2 and more particularly is located over section 7 and the lower end of section 6 thereof. With the filter unit 3 located in position, the lowermost portion of section 7 of column 2 locates in the depression 13 in the upper surface of the filter 12 allowing the filtrate to come into proximity with the liquid inlet 8. As a result, the rate of filtration is enhanced.

Referring to Fig 5, there is illustrated a biomolecule purification assembly 101 for use in obtaining a purified sample of a biomolecule of interest from a suspension (e.g. as obtained by an alkaline lysis procedure) comprising a solution of the biomolecule containing insoluble biological debris. The illustrated assembly 101 comprises a vertically disposed, open-ended column 102 and a filter unit 103.

The column 102 is referenced for convenience as being comprised of body sections 104, 105, 106 and 107. Body section 104 defines an upper cylindrical bore 104a which at its lower end is connected to a downwardly tapering section 105a leading into a lower bore 106a (in body section 106) which is of reduced diameter as compared to bore 104a. At its lower end bore 106a leads into a tapering section 107a defined within the lower section 107 of the column 102.

The lower end of tapering section 107a defines a filtrate inlet for the column.

A shoulder 105b is defined around body section 105.

At the upper end of column 102 there is provided a formation 109 by means of which the column may be mounted on the supporting head of a sample processing apparatus of the type described more fully above.

The filter unit 103 is a two component part and comprises a sleeve 110 within which is housed a filter 111.

The sleeve 110 has a first body portion 112 within which is defined a cylindrical bore 113 having an upper end 113a by means of which the filter unit 103 is mounted on the lower end of column 102. The second body portion 114 has an internal bore 115 which internally tapers from the first body portion 112 to a suspension inlet 116 of the filter unit 103.

At the transition of the bore 113 into bore 116 is a shoulder 117.

The filter 111 (see also Fig 5) comprises a cylindrical head portion 118 and frustoconical body portion 119. At the transition of head portion 118 into body portion 119 is a shoulder 120.

The filter unit 111 locates in sleeve 103 such that the head portion 118 (of the filter) occupies the full cross section of the bore 113 (of sleeve 103), the shoulder 120 (of filter 111) seats on the shoulder 117 (within sleeve 112) and the frustoconical body portion 119 (of the filter) extends into the second body portion 114 (of the sleeve 103) so as to taper therein towards its suspension inlet end 116.

The angle at which the frustoconcical body portion 119 (of the filter 111) tapers is greater than the angle of taper of the bore 115 (of the second body portion

114 of the sleeve 103) so that there is clearance between the outer surface of the frustoconical body portion 119 and the inner, tapering surface of the second body portion 114 of the sleeve 103. This arrangement ensures that there is a relatively large area of the filter exposed to the suspension to be filtered so that the filter unit has a high a capacity for filtration as possible given the overall dimensions of the filtration unit.

Furthermore, the "dead space" within the filter unit 103 (i.e. the space between the outer surface of the frustoconical body portion 119 of the filter 111 and the inner surface of the tapering bored 105) is relatively low whilst still allowing the advantage of the previous paragraph. This "low volume" dead space ensures that there is minimum liquid remaining in the dead space when all of the suspension to be filtered has been drawn up into the filter unit and air is about to enter the column.

At the top of the filter 111 is a generally hemispherical depression 121 which generally serves to improve the flow through the filter. In the assembled biomolecule purification unit, the filtrate inlet for the column 102 is level with the top of the hemispherical depression 121. The depression 121 is however optional and is omitted in the filter illustrated in Fig 7 which is otherwise identical to that of Fig 6.

Reference is now made to Figs 8 and 9 which together schematically illustrate one embodiment of process in accordance with the invention.

As shown in Fig 8, the method is effected using an apparatus incorporating a head arrangement 200 having a line of twelve column supporting heads 201 each connected to a fluid supply line 201a via respectove pumps 202 and valves 203. The fluid supply line 201a is connected to a manifold (not shown) whereby the valves 203 which may selectively provide communication of the heads 201 with a vacuum pump or with liquid supply reservoirs (not shown).

Furthermore as shown in Fig 8, the method is shown as being effected at a number of sequential stations 204 to 208 designated as follows:

Station	Function
204	Lysis/Neutralisation
205	Filtration
206	Magnetic Binding
207	Washing
208	Elution

As described more fully below, a 12 x 8 BioBlock 209 is processed at each of stations 204 and 205. Furthermore 12 x 8 BioBlocks 210 to 212 are also provided at stations 206 to 208 respectively as described in detail below. For the purposes of simplicity only BioBlock 209 is illustrated in Fig 8, and single wells of all BioBlocks 209 to 212 are represented in Fig 9. The head arrangement 200 is illustrated as being movable upwardly and downwardly relataive to the BioBlocks 209 to 212 (as represented by arrow 213) and also relatively through the stations 204 to 208 as represented by arrow 214.

The detailed steps of the method will now be described with additional reference to Fig 9 which illustrates the individual steps of the method.

Station 204 is a lysis and neutralisation station. At this station there is provided the BioBlock 209 containing in each well thereof a sample to be lysed. The sample may for example be a suspension of bacteria containing a plasmid incorporating a DNA sequence of interest. Initially the head arrangement 200 is positioned above the first row of 12 wells (as shown in Fig 8) and is then lowered

relatively thereto (see also Fig 9 (a)). Lysis solution provided in a liquid reservoir (not shown) is supplied through valves 203 so as to be "injected" downwardly and simultaneously into each of the twelve wells (of the first row) of the BioBlock. Each well may be treated with either a single aliquot of lysis solution or multiple aliquots thereof. Subsequently the head arrangement 200 is moved so as to supply lysis solution into each of the twelve wells of the second row of the BioBlock 209 and so on until all ninety six have had lysis solution added thereto. If necessary, the BioBlock 209 may be shaken so as to speed the lysis procedure. Subsequently head arrangement 200 is moved back to the first row of twelve wells (of the BioBlock 209). Neutralising solution, supplied via valves 203, is now passed though each of the heads 201 (again as either single or multiple aliquots) into the first row of twelve wells of the BioBlock 209 (as represented at Fig 9(b)). Subsequently the remaining wells of the BioBlock 209 are treated, twelve at a time, with the neutralising solution.

As the next step, biomolecule purification assemblies 215 (e.g. of the type illustrated as 1 in Fig 1 or 101 in Fig 5) are mounted on each of the column supporting heads 201. More particularly, each biomolecule purification assembly 215 is comprised of a column 216 and its separable filter unit 217. If the biomolecule of interest in the sample is DNA then columns may incorporate a solution of RNase to destroy RNA in the sample if this is likely to be an interferant in the analysis of DNA as isolated by the steps described below.

Filtration is shown as being effected at station 205 to which the head arrangement 200 and BioBlock 209 are moved from Station 204.

At the station 205, the head arrangement 200 is positioned such that the filter units 217 of each of the biomolecule purification assemblies 215 are immersed in the lysate contained in the first twelve rows of the BioBlock 209. Suction is then applied via valves 203 to the column supporting heads 201 whereby liquid is drawn upwardly into the column 216 but solids are prevented from doing so by the filter unit 217. A

stripping comb 218 is now moved into position (see Fig 9 (d)) and head 200 is raised so that the filter 217 is removed from the lower end of column 216 and remains in the well of the BioBlock 209.

In the next step of the method, the head arrangement 200 (supporting the twelve columns 216 each containing filtered lysate held in the column by reduced pressure) is moved to station 206 at which there is a further BioBlock 210 containing in each well thereof a suspension of magnetic particles 219 in a binding medium. Head arrangement 200 is moved relatively downwardly so that the lower ends of the columns 215 are immersed in the suspension of the magnetic particles (see Fig 9(e)). The filtered lysate in the columns 216 is now discharged into the wells of BioBlock 210 for mixing with the suspension the magnetic particles 219 and the mixture is then taken back up into the column 216. There may be at least one subsequent cycle of discharge of this mixture back in to the well of the BioBlock 210 and take up of the mixture back into column 216 to ensure thorough mixing.

A magnet 220 with associated heater 221 is then moved into position. The magnet 220 ensures that the magnetic particles 218 are retained at one position in the column 215 and held at elevated temperature (e.g. 45°C) for a few seconds, e.g. 10 seconds.

The biomolecule of interest will by now have become bound to the particles 219 so that the liquid remaining in column 216 may be discharged therefrom effectively as waste as represented by arrow 222 (Fig 9(f)).

The head arrangement 200 is next moved to station 207 at which a wash solution is introduced via valves 203 into the top of columns 216 so as to wash the particles 219 down into the wells of BioBlock 207. The mixture of particles 219 and wash solution (e.g. 70% aqueous ethanol) may be subjected to repeat cycles of aspiration back into the column and discharge into the wells of BioBlock 207 so as to

ensure thorough washing of the particle 219. The washed particles (with biomolecule bound thereto) are then retained in column 216 and the wash solution discharged into the well of BioBlock 211 (Fig 9(g)).

Finally, head arrangement is moved to station 208 where there is a further BioBlock 212 containing an eluant (e.g. sterile water) which is aspirated into the column of admixture with the particles 219 (released from magnet 220) and release of the bound biomolecule therefrom (Fig 9(h)). During this step the heater 221 may be heated to 60°C. Subsequently the magnet 220 is re-applied and eluant, containing the dissolved biomolecule of interest, discharged into the BioBlock 209 (Fig 9(i).

The twelve columns 216 are now released from the column supporting heads 201 and are discharged to waste (not shown). Subsequently a new set of biomolecule purification assemblies 215 are mounted on the head arrangement 200 which is then positioned over the second row of 12 wells in BioBlock 209 at filtration station 205. The steps described above in relation to Figs 9(c)-(i) are then repeated so as to obtain further samples of the purified biomolecules. Further repeats of this procedure are effected until all ninety six wells of the BioBlock 209 have been processed.

For the purposes of describing the steps of the above process, it has been assumed that head arrangement 200 is translated in the direction of arrow 214 between the various stations 204 to 208. However, in practice, it is possible for there to be no transitional movement in the direction of arrow 214 of the head arrangement 200 but rather for the BioBlocks 209 to 212 to be moved beneath the head arrangement 200 as appropriate.